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Original Research Article

Comparative Research Study of *in vitro* Antioxidant Activity of Leaves Calli and Diverse Geographical Leaves Samples from Ethnomedicinal Herb *Achyranthes aspera* L

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ABSTRACT

Background: *Achyranthes aspera* is an ethnomedicinal herb belongs to family Amaranthaceae. It contains alkaloids, flavonoids, tannins, anthraquinones, saponins, glycosides and unstable oils. The plant is extremely valued by traditional traders and utilized in cure of bronchospasm, internal bleeding, uterine tonic, respiratory infections, cold, chronic wheeze, pain in stomach, weakness, dysentery, ear troubles, pain in head, leucoderma, pneumonia, renal complications, scorpion bite, snake bite and skin diseases.
Aim: Determination of antioxidant activity was performed in leaves calli of and eight different geographical leaves samples of *A. aspera* L.
Material and method: Leaves calli with different concentrations and combination of auxins and cytokinins like 2, 4-Dichlorophenoxyacetic acid (2,4-D), Indole Acetic Acid (IAA), Naphthalene Acetic Acid (NAA) and 6-Benzyl Amino Purine (BAP) were maintained. Three methods DPPH radical scavenging activity, H_2O_2 scavenging activity and ferric reducing power were applied for determination of antioxidant activities of leaves calli and leaves samples.
Result Highest DPPH radical scavenging activity (85.31%), H_2O_2 scavenging activity (75.31%) and ferric reducing power (25.3µg/g) were observed in calli supplemented with 2,4-D (2.0 mg/l) + IAA (0.5mg/l).
Conclusion: Excellent *in vitro* antioxidant activity was given by extract of callus culture on MS medium supplemented with 2,4-D (2.0 mg/l) + IAA (0.5mg/l). So they can be used as natural antioxidants after isolation and purification.

Key words: MS medium, Antioxidant activity, Leaves calli, Auxin, Cytokinin

INTRODUCTION

Usually, the quantity of reactive oxygen species (ROS) and antioxidants formed in the body are evenhanded, but a few inevitable conditions, antioxidant defense mechanism may be insufficient to reimburse the extremely enhanced ROS which is injurious for the body. Similarly, a variety of toxicants operate through the ROS like superoxide anions (O^{2-}), hydroxyl

radicals ($HO^{\cdot}$) and non free radical species such as H_2O_2, singled oxygen (O_2) and nitric oxide (NO) that participate a chief function in beginning of degenerative processes such as cellular damage that may be correlated to several body ailments viz. heart diseases , cancer and aging. Antioxidants are very well occupied in obstacle of cellular damage, Catalase, superoxide dismutase and glutathione peroxidases are a little of the natural antioxidants found in body. They counteract free radicals as the natural by-product of normal cell processes. [1] Because ancient time, *in vitro* studies on medicinal plants and vegetables have revealed growing significance towards natural antioxidants from herbal sources in the form of phytochemicals which use a defensive effect against oxidative stress in biological systems. [2]

Achyranthes aspera L. is found in plains, forests, foot hills, waysides and roadsides. Distributed areas comprise of road sides, abandoned gardens, crops, grasslands, savannah and forest margins. [3]

The plant grows in tropical and warmer regions and is found throughout tropical Asia, Africa, Australia and America. [4] It is situated all over India up to an altitude of 2100 m and in South Andaman Islands, where it mostly grows as a weed on road sides, in vacant agricultural land, especially in uncultivated lands and along the borders of the cultivated fields.

Though nearly all of its parts are utilized in conventional frameworks of solutions, seeds, roots and shoots are the foremost imperative parts which are utilized medicinally. Customarily, the plant is utilized in asthma and hack. There are a lot of pharmacological actions specified by plant like ant periodic, antiasthmatic, hepatoprotective, anti-allergic, expectorant, stomach tonic, purgative, anthelmintic, diuretic, anti-inflammatory, anticataract, antifungal, antibacterial, antidiabetic, hypolipidemic and haematinic and different other critical restorative properties. [4] *A. aspera* L. (Family Amaranthaceae) is a widespread plant richly originated in wastelands. The plant is extremely valued by traditional traders and utilized in cure of bronchopasm, internal bleeding, uterine tonic, respiratory infections, cold, chronic wheeze, pain in stomach, weakness, dysentery, ear troubles, pain in head, leucoderma, pneumonia, renal complications, scorpion bite, and snake bite, skin diseases. [5]

In the present study we have made an effort to determine the total phenolic and flavonoid contents as well as to establish the in vitro antioxidant activity in methanol extract of leaves callus culture and leaves samples of *Achyranthes aspera* L.

MATERIALS AND METHODS

Collection and verification of fresh drugs from different location of India.

The fresh leaves of plant of *Achyranthes aspera* L. were collected from the Herbal Garden, Mewar University, Chittorgarh and from seven other different places of India as given in Table 1 Based on morphological and microscopic examination and compared with the herbarium available at Department of Botany, Mewar University, Rajasthan, leaves of the plant was verified by Prof. (Dr.) B.L. Yadav in Department of Botany, Mewar University, and Chittorgarh. The leaves were dried at room temperature and powdered.

Table 1: List of fresh drug sample collected from different geographical regions of India

S.No	Place	Address (sources)
1	Chittorgarh	Herbal garden of Mewar University, Chittorgarh, Rajasthan
2	Delhi	Herbal garden of Jamia Hamdard, New delhi
3	Madhya Pradesh	Wild region, distt. Sambhag Madhya Pradesh
4	Lucknow	Herbal garden, Faculty of Pharmacy, Integral University, Utter Pradesh
5	Chandigarh	Herbal garden, Panjab University
6	Banaras	Herbal garden of Banaras Hindu university campus, Uttar Pradesh
7	kottayam (Kerala)	Wild region , kottayam (Kerala)
8	Chennai	Wild region, Chennai.

Culture medium and plant growth regulators

Young leaf explants (1-2 cm) were inoculated on MS medium [6] containing 3% sucrose and gelled with 0.8% agar supplemented with various combination of of IAA, 2,4-D and BAP. The pH of the medium was adjusted to 5.8 before gelling with agar and autoclaved for 20 minutes at 121ºC for 15 lbs pressure.

Inoculation of explants

After being sterilized, leaves explants were set on a sterilized petridish with a sheet of millimeter chart paper underneath to permit exact measuring of explants amid dismemberment. The distinctive explants were cut into 5-10 mm pieces with the assistance of a sterile and flared forceps. The explants were exchanged to the culture tubes containing supplement agar media supplemented with diverse development hormones for the acceptance of callus.

Physical conditions of cultures

The cultures were maintained in the culture room at 26 ± 2ºC. The cultures vessels were established under light, generated by Phillips fluorescent tubes (40 W, 220 V) with a light strength of 1400-3300 lux. The comparative humidity (RH) was set aside in the common range (i.e. 60-70%).

After inoculation, culture vessels were located in B.O.D. incubator at 25º C ± 2º C and the culture vessels were contacted to light for 16 hours (having light intensity of 1600 lux) and maintained in the dark for 8 hours, alternatively. These inoculated cultures were evaluated for any growth and in those cultures; growth was established further subculture in to same hormone combinations. v

Sub culturing

Sub culturing was carried out at regular intervals of 21 days. Visual observations of the cultures were taken for every transfer and the effects of different treatments were quantified on the basis of percentage of cultures showing response.

Preparation of methanol extract of calli cultures

Callus cultures derived from leaves explants were air dried at room temperature and ground in a mortar. 0.5 g of the dried powder obtained from each hormonal combination of auxin and cytokinin was extracted with methanol in a water bath at 45°C for 3 h. Whatman filter paper No. 4 was used for filtration of liquid extract. Solvent was evaporated from collected filtrates under vacuum at 40°C. The extraction was rehashed twice. The dried residue was re-dissolved in methanol and utilized for the accumulation of phenolics and flavonoid contents and determination of antioxidant activities.

Evaluation of total phenol content [7]

Material and Reagent

Reference standard, for flavonoid Rutin and for phenol compound gallic acid were received from M/s Normal cures Pvt. Ltd. Bangalore, India. Each chemicals and reagents utilized were of explanatory rating and acquired from Merck Chemicals, India.

Preparation of methanol extracts of all samples: one gm of leaf and callus sample were dried and powdered then refluxed in 50 ml of methanol for two hours. Collected filtrate was filtered and heated till residue formation .The weight of residue was measured and reconstituted in methanol.

Reagents

- 10% F.C. reagent solution in distilled water
- 1M Na_2CO_3 (sodium carbonate) solution in distilled water
- Standard (Gallic acid) 1 mg/ml solution in methanol
- Dilution of standard Gallic acid: 50 µg/ml, 100 µg/ml and 150 µg/ml in methanol

Samples preparation: 10 mg / ml methanol leaf extract and 8 mg/methanol calli extracts were made. 0.5 ml sample was poured to mixture of 5 ml F.C compound and 4 ml volume of Na_2CO_3 then took absorbance on 765 nm after 15 minutes.

Preparation of Standard: 0.5 ml of each standard dilution was mixed to mixture of 5 ml volume F.C. reagent and 4 ml Na_2CO_3 solution, then took absorbance at 765 nm after 15 minutes.

Blank solution: Methanol 5 ml solvent and 5 ml F.C. reagent were taken and added to 4 ml Na_2CO_3 solution.

4.8.2 Evaluation of entire flavonoid content [7]

Reagents

- mg/ml $AlCl_3$ (aluminium chloride) solution in distilled water
- 1M CH_3COONa (sodium acetate) solution in distilled water
- Standard (Rutin) 1 mg/ml solution in methanol
- Dilution of standard Rutin: 10 µg/ml to 100 µg/ml in methanol

Samples preparation: 100 mg /ml solution for leaves extract and calli extract were included in methanol. Half ml of sample was added to 1.5 ml methanol. Combination of 0.1 ml volume of $AlCl_3$ and 1/10 ml volume of CH_3COONa reagents were poured to above solution. After that 2.8 ml volume distilled water mixed to solution and kept for half hour after that absorbance was maintained at 415 nm.

Preparation of Standard: 0.5 ml volume of standard dilution were taken and added to 1.5 ml methanol. After that 0.1 ml volume of $AlCl_3$ and 0.1 ml of CH_3COONa reagents were poured to above solution and then added 2.8 ml Distilled water and kept for half hour after that absorbance was taken at 415 nm.

Blank solutions: two ml methanol was added to mixture of 0.1 ml volume of aluminium chloride and 0.1 ml volume of CH_3COONa and then added to 2.8 ml volume purified water.

Methods for antioxidant activity

DPPH liberated radical rummaging activity

0.1 mM DPPH was mixed in methanol and 1 ml mixture was included to 3 ml alcohol extract at one concentration (500 µg/mL). Butyrate hydroxytoluene (BHT) was utilized as a positive standard. Discoloration was calculated on 517 nm after hatching for 30 min. Estimations were taken at slightest in triplicate. The capacity to rummage the DPPH radical was calculated utilizing the taking after condition: DPPH rummaging impact (%) = $[A_{DPPH} - A_S / A_{DPPH}]$ x100 where, A_{DPPH} is the UV absorbance of the DPPH arrangement and A_S is the UV absorbance of the arrangement when the test extricate is included. The extract concentration giving 50% restraint of radical-scavenging movement (IC_{50}) was calculated and communicated as mg/mL, d.w. [8]

Ferric diminishing control determination

2.5 ml phosphate buffer for pH maintaining (200 mM and pH 6.60) and 500 µg/mL alcohol extracts were blended with and 2.5 ml 1% potassium ferricyanide. At that point the blend was brooded at 50 °C with twenty minutes. 2.5 ml 10% dilute trichloroacetic acid was included to over blend and centrifuged at 10000 revolutions per minute for ten minutes. 5 ml of the top surface was poured with distill water (5 ml) and 0.1% $FeCl_3$ (1 ml). The absorbance of the response blend was measured at 700 nm. The ultimate comes about were communicated as µg ascorbic acid counterparts / g based on dry weight of the extract. [9]

Hydrogen peroxide rummaging activity

Solution of H_2O_2 (43 mM) was made in buffer phosphate (0.10 M, pH 7.4). Extricates at concentration 50 µg/mL broken up in 3.4 mL phosphate buffer were included to a H_2O_2 arrangement (600µL). The absorbance of the response blend was recorded at 230 nm. The extricate concentration giving 50% of H_2O_2 rummaging movement (IC_{50}) was calculated and communicated as µg/mL based on test dry weight. [10]

Statistical analysis

The experiments were performed utilizing Completely Randomized Design (CRD). Triple readings were taken in each experiment. Data are given as means ± standard deviation (SD). Analysis of

variance and significant differences among means were tried by one-way ANOVA using the COSTAT computer package (Cohort Software, 1989). The least significant difference (LSD) at $P \leq 0.05$ level was estimated. Correlation coefficients (R^2) from regression analysis between total phenolic, flavonoid contents and antioxidant activities were also estimated.

RESULTS

Inoculation and selection of suitable phytohormonal combinations for development of callus from eaves explants of *A. aspera* L.

Proper phytohormonal combinations were selected in this experiment for the initiation of callus on leaves explants of *A. aspera* L. Assortment of diverse combinations and concentrations of phytohormones IAA, BAP and NAA were incuded in the MS medium.

After various efforts four hormone combinations were observed with high intensity callus formation and 100 % callus initiation response.

HC1: MS + 2, 4-D (1.7 mg/L) + BAP (0.5 mg/L)

HC2: MS + 2, 4-D (0.5 mg/L) + BAP (0.5 mg/L) + IAA (0.2 mg/L)

HC3: MS + 2, 4-D (1.5mg/L) + NAA (0.2 mg/L)

HC4: MS + 2, 4-D (1.5 mg/L) + NAA (0.5 mg/L)

HC1 **HC2** **HC3** **HC4**

Figure 1: Development and maintenance of leaves calli of *Achyranthes aspera* L.

Total phenolics and flavonoid content in leaves calli and different geographical leaves samples.

Total phenolic content of different leaf samples and calli sample were estimated using UV spectro photometric method at 765 nm. The standard calibration curve for gallic acid was established linear in the range of 50 –150µg/ml with $r^2 = 0.997$ and regression equation Y = 0.0002X + 0. 131. Table 2 shows the content of total phenol (3.71 %) was found highest in the calli supplemented with 2,4-D (1.5 mg/L) + IAA (0.5mg/L).

Table 2 : Entire phenolic contents in leaf extract samples and dried calli samples.

Samples name	*Absorbance	*Concentration in µg/ml	% w/w
Chittorgarh	0.328 ± 0.4515	980 ± 0.6520	0.980 %
Delhi	0.309 ± 0.6572	840 ± 0.5019	0.840 %
Madhya Pradesh	0.303 ± 0.5617	810 ± 0.8717	0.810 %
Lucknow	0.314 ± 0.7227	915 ± 0.3132	0.915 %
Chandigarh	0.332 ± 0.3421	1005 ± 0.3575	1.00 %
Banaras	0.339 ± 0.6731	1040 ± 0.2793	1.04 %
Kottayam	0.384 ± 0.9839	1490 ± 0.5972	1.49 %
Chennai	0.364 ± 0.8441	1165± .2077	1.16 %
HC1	0.712 ± 0.3426	2905± 0.5733	2.90 %
HC2	0.703 ± 0.4326	2810± 0.4372	2.81 %
HC3	0.817 ± 0.3452	3430±0.5489	3.43 %
HC4	0.873 ± 0.7654	3710±0.6345	3.71 %

(*= Mean ± SEM of 3 replication)

The entire flavonoid content of diverse leaves samples and calli samples was estimated utilizing UV spectro photometric method with Rutin as standard at 415 nm. The standard calibration graph was originated linear in the series of 10 – 100 µg/ml with r^2 =

0.999 and regression equation with Y = 0.005X + 0.339. Table 3 shows highest flavonoid content (1.38 % w/w) was created in the calli supplemented with 2,4-D (1.5 mg/L) + IAA (0.5mg/L). The rest of samples contained flavonoid content in between 1.22% – 0.70% w/w.

Table 3: Total flavonoid contents in leaf extract samples and dried calli samples.

Samples name	*Absorbance	*Concentration in µg/gram	% w/w
Chittorgarh	0.298 ± 0.4515	8.2 ± 0.6520	0.82%
Delhi	0.302 ± 0.6572	7.4 ± 0.5019	0.74 %
Madhya Pradesh	0.304 ± 0.5617	7.0 ± 0.8717	0.70 %
Lucknow	0.295 ± 0.7227	8.8 ± 0.3132	0.88 %
Chandigarh	0.290 ± 0.3421	9.8 ± 0.3575	0.98 %
Banaras	0.292 ± 0.6731	9.4 ± 0.2793	0.94 %
Kottayam	0.288 ± 0.9839	10.2 ± 0.5972	1.02 %
Chennai	0.286 ± 0.8441	10.6 ± .2077	1.06 %
HC1	0.280 ± 0.3426	11.8 ± 0.5733	1.18 %
HC2	0.278 ± 0.4326	12.2 ± 0.4372	1.22 %
HC3	0.275 ± 0.3452	12.8 ± 0.5489	1.28 %
HC4	0.270 ± 0.7654	13.8 ± 0.6345	1.38 %

(*= Mean ± SEM of 3 replication)

From table 4 data it was found that the antioxidant activity of leaves sample and leaves calli of *A. aspera* L. was determined using the DPPH liberated radical rummaging action, ferric reducing power determination and hydrogen peroxide rummaging activity methods. Table 4 shows maximum radical scavenging activity (84.39 %) with IC_{50} value (0.872 mg/ml), ferric reducing activity(22.52 µg/g) and hydrogen peroxide rummaging activity (75.06%) with IC_{50} (76.53 µg/ml) were observed in methanol extract of leaves calli supplemented with MS + 2, 4-D (1.5 mg/L) + NAA (0.5 mg/L). Minimum radical scavenging activity (70.63 %) with IC_{50} value (0.513 mg/ml), ferric reducing activity(18.40 µg/g) and hydrogen peroxide rummaging activity (64.63 %) with IC_{50} (63.65 µg/ml) were estimated in methanol extract of Chittorgarh sample.

Table 4: Comparative *in vitro* antioxidant action of of calli and diverse geographical leaves samples of *A. aspera* L.

Sample extract	*DPPH scavenging activity (%) (mean ± SEM)	*IC50 values (mg/mL, d.w.) of DPPH scavenging activity	*Ferric reducing power(µg/g, dw)	*Hydrogen peroxide scavenging activity (%)	*IC50 values (µg/mL, d.w.) of H_2O_2 scavenging
Chittorgarh	70.63±0.3452	0.513±0.5489	18.4±0.9834	64.63±0.3076	63.65±0.0765
Delhi	75.31±0.6732	0.601±0.9834	18.8±0.6875	67.34±0.4590	66.43±0.3054
Madhya Pradesh	79. 87±0.2929	0.711±0.8065	19.2±0.3564	69.98±0.1098	68.87±0.2054
Lucknow	74.31±0.5698	0.593±0.5042	18.5±0.6754	66.54±0.3076	65.43±0.4021
Chandigarh	73.72±0.3290	0.553±0.2309	18.01±0.2389	65.98±0.2390	65.13±0.3076
Banaras	77.65±0.5490	0.709±0.5011	19.02±0.9043	67.53±0.4053	67.43±0.1012
Kottayam	80.89±0.9087	0.754±0.6021	20.11±0.7832	70.24±0.3408	69.87±0.1132
Chennai	77.31±0.5602	0.702±0.8043	19.01±0.5367	67.14±0.2075	66.54±0.4562
HC1	82.13±0.4031	0.821±0.9032	20.34±0.0876	72.21±0.4076	70.65±0.9987
HC2	79.99±0.9065	0.731±0.4087	19.4±0.1089	70.03±0.1076	70.08±0.1156
HC3	81.36±0.5396	0.811±0.7043	20.28±0.0874	72.09±0.9863	72.54±0.2343
HC4	84.39±0.3480	0.831±0.3208	21.9±0.4576	73.65±0.2096	73.98±0.6324
Control	87.37±0.7654	0.872±0.3434	22.52±0.0863	75.06±0.3452	76.53±0.9823

(*= Mean ± SEM of 3 replication)

The high DPPH activity could be correlated with high phenolics content. Absorbance of DDPH radical decrease with high phenol content. DDPH works as an established free radical in extract solution that simply accepts an electron or hydride radical and transformed to a constant diamagnetic molecule. By reacting with appropriate reducing agents DPPH radicals transformed into the corresponding hydrazine.

Hydrogen peroxide, although not a radical species play a role to contribute oxidative stress. The generation of even low levels of H_2O_2 in biological systems may be important. Naturally-occurring iron complexes within the cell supposed to react with H_2O_2 *in vivo* to create highly reactive

hydroxyl radicals and this may be the beginning of many of its noxious effects [11] use it may generate hydroxyl radical in the cells. [12]

So, elimination of H_2O_2 is very significant for safety of food systems. Scavenging of H_2O_2 by extracts may be attributed to their phenolics, which can donate electrons to H_2O_2, thus neutralizing it to water.

The huge quantity of antioxidants in the extracts from *A. aspera* L. were responsible in the reduction of Fe3+and Fe2+ by providing an electron. The quantity of $Fe3^+$and $Fe2^+$ can be expressed through the blue colour form and estimated at 700nm. [13] There is the important connection between the phenolic compounds and antioxidant action. [14] Reducing power is possible by the existence of the elevated phenolic compounds of the plant. [15]

CONCLUSION

Based on the outcome obtained in the present study, it was accomplished that regenerated culture from leaf explants of *Achyranthes aspera* L. supplemented with 2,4-D (1.5 mg/l) + IAA (0.5mg/l) contain elevated extent of total phenolics and flavanoids than intact plant leaves. They provide considerable antioxidant activities to hydroxyl radical, superoxide radical, and DPPH radical. Methanol extracts of leaves calli with different hormonal combination have different antioxidant activities. There was found a important and linear relationship between the antioxidant activity and the content of flavonoids. Thus, the extract from callus culture of *A. aspera* L. could be used as an antioxidant herb for adjuvant therapy. As the synthetic antioxidant Butyl Hydroxy Toluene was forbidden being used in food due to its side effects on human, development of the natural antioxidants was meaningful and prospective. In this study, antioxidant activities of leaves calli from *A. aspera* L. was found more than extract of intact leaves of plant. So they can be used as natural antioxidants after isolation and purification.

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REFERENCES

1. Matkowski A, Piotrowska M. Antioxidant and free radical scavenging activities of some medicinal plants from the Lamiaceae. Fitoterapia. 2006; 77: 346- 353.
2. Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. Journal of Agriculture and Food Chemistry. 1998; 46: 4113-4117.
3. Barua CC, Talukdar A, Begum SA, Pathak DC, Sarma DK, Borah RS and Gupta A. *In vivo* wound-healing efficacy and antioxidant activity of *Achyranthes aspera* in experimental burns. Pharmaceutical Biology. 2012; 50: 892-899.
4. Srivastav S, Singh P, Mishra P, Jha KK and Khosa RL. *Achyranthes aspera*-An important medicinal plant: A review. Journal of Natural Product and Plant Resources. 2011; 1: 1-14
5. Khare P and Saxena M. Ethno medicinal study for human healthcare among the people of Chhatarpur District, Madhya Pradesh, India. Plant Archives. 2007. 7: 899-901.
6. Murashige T, Skoog F. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Plant Physiology. 1962; 15: 473-497.
7. Pourmorad F, Hosseinimehr SJ and Shahabimajd N. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. African Journal of Biotechnology. 2006; 5: 1142-1145.
8. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958; 181: 1199–1200.
9. Zhao *et al.* Evaluation of antioxidant activities and total phenolic contents of typical malting barley varieties. Food Chemistry. 2008; 107: 296–304.

10. Shon *et al*. Antioxidant and free radical scavenging activity of methanol extract of chungkukjang. Journal of Food Composition and Analysis. 2007; 20: 113–118.
11. Miller HE, Rigelhof F, Marquart L, Prakash A, Kanter M. Antioxidant Content of Whole Grain Breakfast Cereals, Fruits and Vegetables. Journal of the American College of Nutrition. 2010; 19: 312-319.
12. Halliwell B. The antioxidant paradox. Lancet 2000; 355: 1179-1180.
13. Akinpelu DA, Aiyegoro OA, Okoh AI. The *in vitro* antioxidant property of methanolic extract of *Afzelia africana* (Smith.). Journal of Medicinal *Plants* Research. 2010; 4: 2001-2027.
14. Thirugnanasampandan R, Mahendran G, Narmatha Bai V. Antioxidant properties of some medicinal Aristolochiaceae species. *African Journal of Biotechnol*ogy. 2008; 7: 357-361.
15. Yildirim A, Mavi A, Kara AA. Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. Journal of Agriculture and Food Chemistry. 2001; 49: 4083-4089.

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